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A new species of *Pseudochalcura* (Hymenoptera, Eucharitidae), with a review of antennal morphology from a phylogenetic perspective

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Abstract

Pseudochalcura alba Heraty & Heraty, **sp. n.** is described from Santiago del Estero and Catamarca provinces in northwestern Argentina. The male and female have long dorsal rami on all of the flagellomeres basal to the terminal segment, which is a unique feature within the genus and shared only with some species of *Rhipipalloidea*. Antennal modifications are compared across the Stilbula clade, of which all are parasitoids of Camponotini (Formicinae). A phylogenetic hypothesis for the group is proposed based on an analysis of 28S and 18S sequence data for 28 species. Ramose antennae are derived independently in both males and females across the clade, but with fully ramose female antennae restricted to the New World *prolata* group of *Pseudochalcura* and to some species of the Old World genus *Rhipipalloidea*. A sister group relationship between these genera is proposed based on both morphological and molecular data. Female antennae in other species of these genera, and other genera in the clade are at most dorsally lobate or serrate, but more commonly cylindrical. Monophyly of species of *Obeza* and *Lophyrocera* is supported and linked to a behavioral trait of oviposition into fruits as opposed to flower heads or leaf buds. Within the Stilbula clade, a dichotomy between New and Old World taxa suggest relatively recent post-Miocene exchanges across the Northern Hemisphere.

Keywords

Eucharitidae antenna phylogeny

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Introduction

Eucharitidae (Hymenoptera: Chalcidoidea) are parasitoids of ant pupae (Clausen 1940, Heraty 2002). A variety of methods are employed to gain access to the ant brood, but all involve oviposition outside of the nest and the active transport of the first-instar planidial larva. The Stilbula clade is a distinct group within the Eucharitini (Eucharitidae: Eucharitinae), with all known host records from Camponotini (Formicinae) (Clausen 1940, Heraty 2002). Oviposition within this group is either in large egg masses that resemble fruit, or into the skin of fruits themselves, thus introducing larvae into the foraging proximity of their hosts (Clausen 1940; Heraty and Barber 1990, Torréns et al. 2008). Based on morphology, the clade is either monophyletic or paraphyletic (Heraty 2002), but monophyletic for either molecular data alone (28S and 18S) or in combination with morphological data (Heraty et al. 2004). *Pseudochalcura* exemplify the behavior typical of the clade and are the focus of this paper, along with the description of a species with unusual antennal features for both the genus and family.

Pseudochalcura are comprised of 10 recognized species that range in the New World from Chile and Argentina to the Yukon and Alaska, although several new species have been discovered since their first revision (Heraty 1986, JMH unpublished). *Pseudochalcura gibbosa* (Provancher) oviposit masses of 1000–2000 eggs into leaf buds of various Ericaceae and Malvaceae (Cook 1905; Pierce and Morrill 1914; Clausen 1940; Heraty & Barber 1990). Eggs of this species overwinter in the leaf bud and likely fall to the ground as the buds expand and the bud scales drop in the spring. Simultaneous hatching of the egg mass upon stimulation by the host ant is seen as a means of both attracting ants (recruitment) and for gaining transport back to the ant brood as a food resource (Heraty and Barber 1990). First-instar planidial larvae of *P. gibbosa* initially attack larvae of *Camponotus novaeboracensis* (Fitch), with development completed by one to three individuals on a single host pupa (Wheeler 1910; Heraty and Barber 1990). The only other confirmed host record is for *P. nigrocyanea* Ashmead from Brazil, for which adults were observed exiting from a nest of *Camponotus* and ovipositing into flower buds of an unidentified Rosaceae (Heraty unpublished). Deposition of egg masses into flower buds is shared with *Stilbula* (Clausen 1940) and *Substilbula* (Heraty, unpublished), and is likely a plesiomorphic behavior for the Stilbula clade with oviposition into fruit regarded as a derived behavior (Heraty and Barber 1990; Torréns et al. 2008).

The form of the antennal flagellum in Eucharitidae is highly variable, ranging from cylindrical or lobate, serrate or ramose in both males and females (Heraty 2002). Antennae may be simple to serrate, with single long rami on the flagellomeres, or with paired elongate rami as found in some species of *Saccharissa* and *Chalcura* (Heraty 2002). Antennal rami can be either cylindrical, flattened, or even antler-like as in *Tricoryna alcicornis* Bouček (Bouček 1988; Heraty 2002). Some genera even have an increased number of flagellomeres, with *Eucharissa erugata* Heraty having as many as 18. Morphological diversity in the antennal flagellum is restricted to the tribe Eucharitini, with all other Eucharitidae (Oraseminae, Gollumiellinae, and Psilocharitini [Eucharitinae]) having simple, cylindrical flagellomeres. Ramose antennae are independently

developed in various clades within Eucharitini, and, in males, often fixed within larger groups such as in virtually all of the poneromorph parasitoids. Similar morphological changes are rare within females, which often have at most dorsally serrate antenna. The Stilbula clade is unique in its extreme level of antennal variation which is paralleled in both males and females. Also notable is a similar amount of antennal variation within *Pseudochalcura*. Only within the Stilbula clade do the female antennae have the same diversity of form as the males. The variation has not been examined previously from a phylogenetic perspective in a closely related group to observe whether changes are homoplastic or synapomorphic. Herein we describe the extreme variation possible in a new species and an undescribed species of *Rhipipalloidea*, as well as resolution of these changes on a phylogeny proposed for members of the Stilbula clade. We also discuss changes in their behavior and correlated biogeographic considerations.

Materials and methods

Terms for descriptive purposes follow Heraty (2002). Images were obtained using GT-Vision® Ento-Vision software operating on a Leica M16 zoom lens linked to a JVC KY-F75U 3-CCD digital video camera. Images were enhanced with Adobe Photoshop CS2. Museums for deposition include the Entomology Research Museum, University of California Riverside (UCRC) and the Instituto Fundación Miguel Lillo, Tucumán, Argentina (IFML). Images for antenna of other species of *Pseudochalcura* and *Rhipipalloidea* (Figs 10–22) are based on a variety of material, with information on locality and specimen deposition available from the senior author upon request. The undescribed species of *Rhipipalloidea* is from Tenompok (Sabah, Malaysia) and is deposited in the Bishop Museum. Illustrations of antennae in Fig. 23 were redrawn from Heraty (1985, 2002).

Molecular Analyses – Thirty-four individuals were sequenced representing 28 species of Eucharitinae (Eucharitidae), with three species of *Pseudometagea* selected as the outgroup (Table 1). *Pseudometagea* are parasitoids of Lasiini (Formicinae) and restricted to the Nearctic Region and Central America (Heraty 1985). The remaining 7 genera are included in the Stilbula clade of parasitoids of Camponotini (Heraty 2002). Conspecific individuals with identical sequence were eliminated from the analysis but indicated on Fig. 23. The complete matrix included 30 terminal taxa. Voucher specimens, except where indicated as "not available", are deposited at UCRC (Table 1).

Adults were removed from 95–100% EtOH and dried in open Eppendorf tubes at 32°C for 30 minutes prior to either a phenol-chloroform extraction (Campbell et al. 1993) or Chelex® extraction (Walsh et al. 1991). Three ribosomal transcript regions were sequenced (28S-D2, 28S-D3 and 18S helices E17–E35, after Ouvrard et al. 2000). The following forward (F) and reverse (R) primer combinations were used: 28S-D2, (F) 5'-CGT GTT GCT TGA TAG TGC AGC-3' or (F) 5'- CGG GTT GCT TGA GAG TGC AGC-3' and (R) 5'-TCA AGA CGG GTC CTG AAA GT-3' (base numbers 3551–4057 of *Drosophila melanogaster*, Hancock et al. 1988); 28S-D3,

Table 1 List of taxa, with DNA voucher codes (p = primary; s = secondary), collection localities, UCR museum specimen identifiers (na = no voucher), and Genbank accession numbers.

Species	Voucher	Locality	Museum #	28S-D2	28S-D3	18S
Leurocharis serricornis Heraty	D0166	Australia: SA	091409p	AY552219	AY552219	AY552296
Lophyrocera pretendens (Walker)	D0634	Colombia	091397p	AY552227	AY552227	AY552304
Lophyrocera sp. 1 ?*pretendens*	D1301	Brazil: RO	000729p	GQ331911	GQ331911	GQ331909
Lophyrocera sp. 2 (near *variabilis*)	D2496	Argentina: SE	184046p	GQ331912	GQ331912	n/a
Lophyrocera variabilis Torréns et al.	D2325	Argentina: TU	184043s	GQ331913	GQ331913	n/a
Lophyrocera variabilis	D2553	Argentina: TU	161496p	GQ331914	GQ331914	n/a
Obeza floridana (Ashmead)	D0024	USA: FL	n/a	AY672976	n/a	n/a
Obeza grenadensis (Howard)	D0358	W.I.: St. Lucia	n/a	AY552225	AY552225	AY552302
Obeza sp. 1	D1074	Argentina: SA	091454p	AY552226	AY552226	AY552303
Obeza sp. 2	D2493	Argentina: MI	184047p	GQ331915	n/a	n/a
Obeza sp. 3	D0631	Colombia	092157s	AY672977	AY672977	n/a
Pseudochalcura alba H&H female	D0349	Argentina: LR	091260p	AY672973	AY672973	n/a
Pseudochalcura alba male	D2511	Argentina: LR	161499p	GQ331916	GQ331916	n/a
Pseudochalcura americana (Howard)	D0635	Colombia	091425p	AY552217	AY552217	AY552294
Pseudochalcura gibbosa (Provancher)	D0910	USA: WY	091401s	AY552218	AY552218	AY552295
Pseudochalcura nigrocyanea Ashmead	D0179	Brazil: RO	161585s	AY672972	AY672972	GQ331910
Pseudochalcura pauca Heraty	D2488	Argentina: SA	184050p	GQ331917	n/a	n/a
Pseudochalcura prolata Heraty	D2494	Argentina: SE	184051p	GQ331918	GQ331918	n/a
Pseudochalcura sp. 1	D1788	USA: FL	184052s	GQ331919	n/a	n/a
Pseudochalcura sp. 2 (nr *frustrata* H.)	D2495	Argentina: SE	184053p	GQ331920	GQ331920	n/a
Pseudometagea bakeri Gahan	D0322	Canada: SK	091253s	AY672971	AY672971	n/a
Pseudometagea montana (Ashmead)	D0321	Canada: ON	091473p	AY552216	AY552216	AY552293
Pseudometagea schwarzii (Ashmead)	D0274	USA: MN	091457s	AY552215	AY552215	AY552292
Rhipipalloidea mira Girault	D1311	Australia: QLD	091861p	GQ331921	GQ331921	n/a
Stilbula quinqueguttatta (Girault)	D0644	Australia: QLD	091439s	AY552222	AY552222	AY552299
Stilbula sp.	D2692	Nigeria: Ondo	000315p	GQ331923	GQ331923	n/a
Stilbula ussuriensis Gussakovskiy	D0287	Russia: Prim. Kr.	091417p	AY552221	AY552221	AY552298
Stilbula ussuriensis	D0923	Russia: Prim. Kr.	103442s	GQ331922	GQ331922	n/a
Stilbuloida doddi (Bingham)	D0660	Australia: NT	091438p	AY552224	AY552224	AY552301
Stilbuloida doddi	D1314	Australia: QLD	092244p	GQ331924	GQ331924	n/a
Stilbuloida ?*doddi*	D0642	Australia: QLD	091391s	AY552223	AY552223	AY552300
Substilbula pallidiclava (Girault)	D0965	Australia: QLD	091416p	AY552220	AY552220	AY552297
Substilbula pallidiclava	D0966	Australia: QLD	n/a	GQ331925	GQ331925	n/a
Substilbula pallidiclava	D1011	Australia: QLD	103430p	GQ331926	GQ331926	n/a

(F) 5'-GAC CCG TCT TGA AAC ACG GA-3' and (R) 5'-TCG GAA GGA ACC AGC TAC TA-3' (base numbers 4046–4413 of *D. melanogaster*); 18S helices 17–35, (F) 5'-AAA TTA CCC ACT CCC GGC A-3' and (R) 5'-TGG TGA GGT TTC CCG TGT T-3' (base numbers 452–1310 of *Hackeriella veitchi*; Ouvrard et al. 2000). PCR protocols follow Babcock et al. (2001). PCR products were purified using GeneClean® PCR Purification Kits (Q-BIOgene). Both top and bottom strands were sequenced at either the San Diego Microchemical Core Facility or the UCR Genomics facility. Sequences were deposited in GenBank (Table 1). Sequences were aligned visually according to the alignment protocol used by Heraty et al. (2004). The alignment consisted of 1767 bases, with partitions: D2: 645 bases (59 parsimony informative), D3: 338 bases (25 PI), 18S: 784 bases (13 PI).

Analysis – Parsimony analyses were implemented using PAUP*4.0 b10 (Swofford 2002) using 500 random-addition heuristic searches and TBR branch swapping. Uninformative sites were included in all analyses. Gaps were treated as missing. Trees were condensed for branches with a minimum length of zero. Successive approximation character weighting was applied to the resulting trees using the maximum value of the rescaled consistency index and a base weight of 1000, with the resulting tree rescaled to unity character weights and compared in length to the most parsimonious trees (Carpenter 1988; Heraty et al. 2004). Bootstrap values for parsimony analyses were evaluated with 1000 random replicates and 2 random addition searches per replicate. In addition, a second partitioned likelihood analysis was performed using RaxML with three partitions and 200 rapid bootstrap replicates (Stamatakis et al. 2008) as implemented on the CIPRES server (http://www.phylo.org/sub_sections/portal/). Each partition was analyzed under a separate GTR+I+Γ model as applied in Heraty et al. (2004).

Description

Pseudochalcura alba **Heraty & Heraty, sp. n.**
urn:lsid:zoobank.org:act:97405532-D55F-449C-9303-2F8BDFE1137E
Figs 1–9

Diagnosis. This is the only species of *Pseudochalcura* in which the male has flagellomeres 1–9 each with a long branch, the female has a long branch on flagellomeres 1–7, and both sexes have a 10-segmented flagellum. This species is placed in the *condyla* group of species based on the bare wing veins, sculptured (strigate) gastral sternite 1 in the female, absence of a metatibial spur, and ramose female antenna (to couplet 11 in Heraty 1986). No other member of this group has a 12-segmented female antenna or completely ramose male antenna. The closest species is considered to be *P. prolata* Heraty (Argentina: Chaco Province), which has the basal 5–7 flagellomeres of the female antenna each with a stout branch, and a maximum of 8–11 antennal segments (male unknown). The basal branch of the female of *P. prolata* is stouter and 5.4 times as long as the basal length, as compared to 6.2 times in *P. alba*.

Male (Holotype). Length 2.35 mm. Black with faint metallic luster; scape, pedicel, mandible, petiole, and basal quarter of femora light brown; antennal flagellum and rest of legs white. Wings hyaline, stigma clear.

Head 1.79 times as broad as high (Fig. 4). Posterior ocellar line (POL) 2.8 times lateral ocellar line (LOL); POL 3.3 times ocellar ocular line (OOL). Frons and face irregularly costate (Fig. 4); clypeus and supraclypeal area smooth; genal bridge emarginate behind the mouthparts. Eyes separated by 2.2 times their height. Malar space 1.1 times height of eye, malar depression absent. Apical tooth of mandible long and overlapping opposing genal area. Labrum not observed. Antenna 12-segmented (Fig. 4); scape short and cylindrical, not reaching to median ocellus; all flagellomeres but the last with long branches ranging from 7.4 to 12.3 times as long as basal length, flagellomere branches alternating in origin from the base and slightly flattened; apical flagellomere unbranched and 3.6 times longer than broad; flagellomeres with dense short setae, and multiporous plate sensilla small and recessed into depressions of the antennal wall.

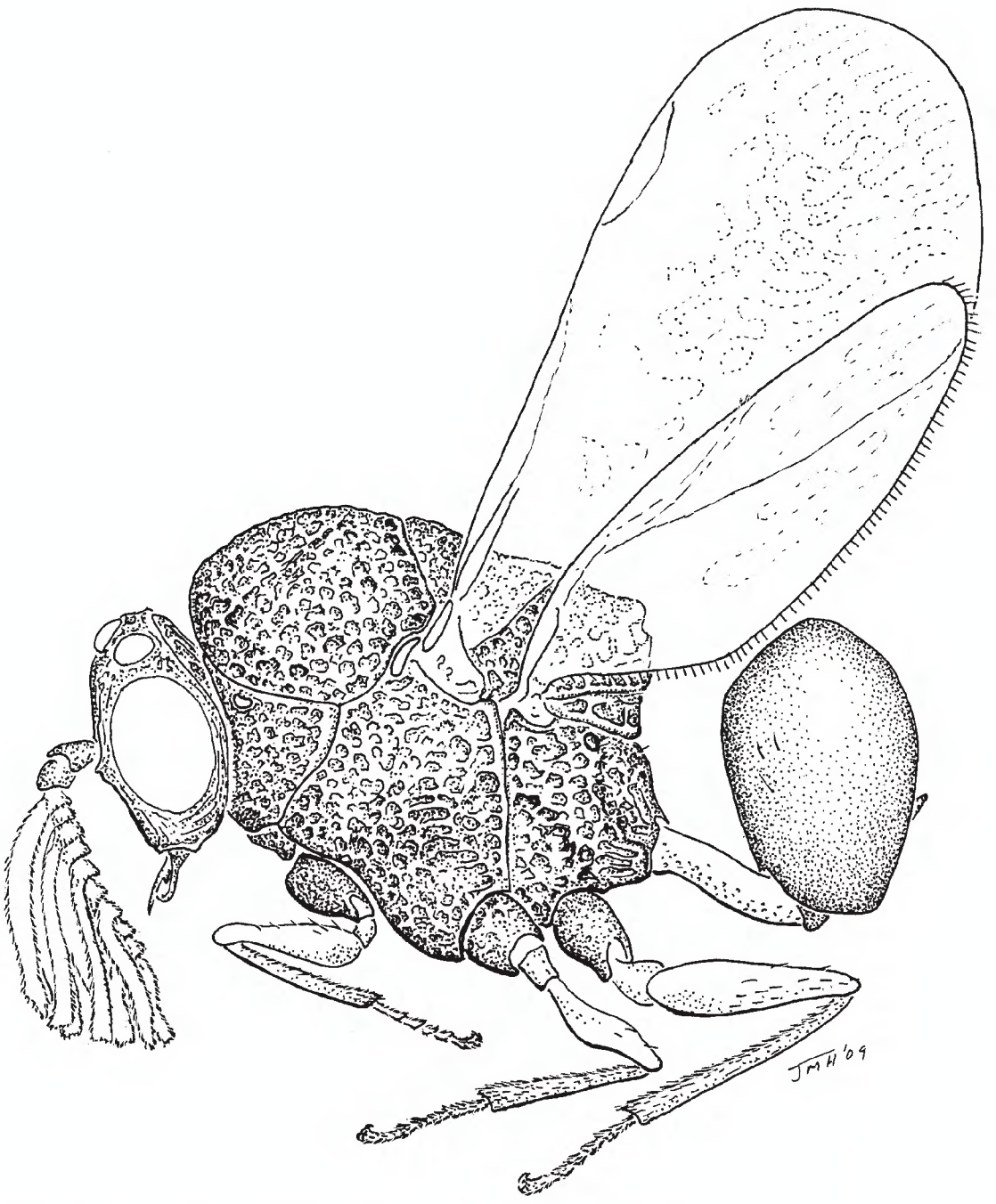
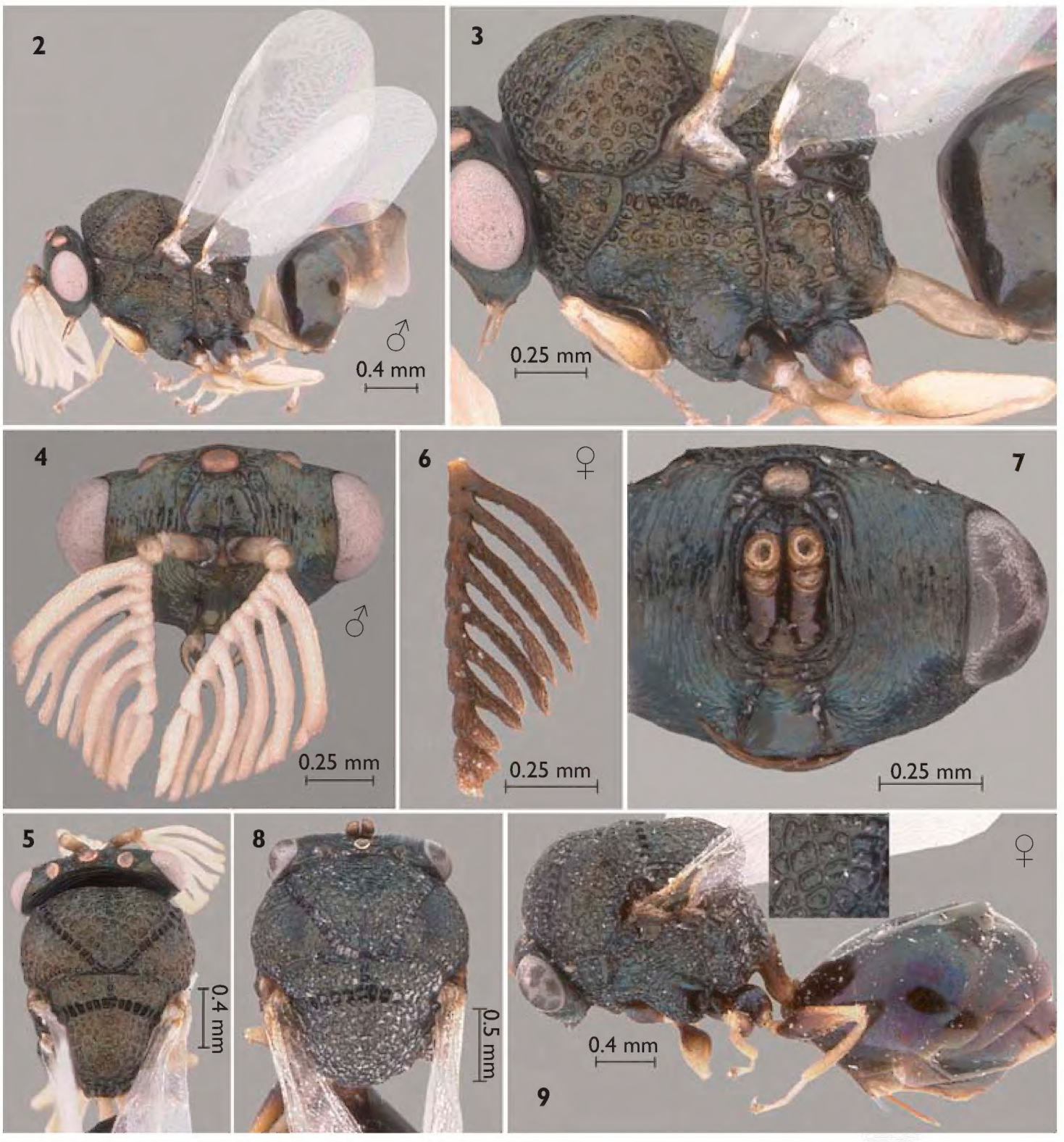


Figure 1. *Pseudochalcura alba* sp. n., male holotype: habitus, lateral.

Mesosoma mostly with areolate sculpture, interstices broad with verrucose sculpture (Figs 2, 3, 5). Mesoscutum 2.0 times as broad as long; scutoscutellar sulcus transverse and deeply foveate; scutellar-axillar complex 1.4 times as long as maximum width of scutellum. Propodeal disc slightly rounded with broad alveolate sculpture medially and a few scattered setae dorsally, disc laterally relatively smooth; callus and metapleuron alveolate. Femoral groove deeply impressed. Proepisternum rugulose, becoming smooth apically. Mesocoxa weakly sculptured and lacking a lateral carina; metacoxa mostly smooth, laterally with small scattered pits. Legs stout (Figs 1, 2, 3), metafemur expanded medially, 3.5 times as long as broad; with short sparse appressed setae later-



Figures 2–9. *Pseudochalcura alba* sp. n.: **2–5** male holotype: **2** lateral habitus **3** mesosoma, lateral **4** head, frontal **5** mesosoma, dorsal **6–9** female paratype: **6** antenna, lateral **7** head, frontal **8** mesosoma, dorsal **9** habitus, lateral. Inset is magnification of mesoscutal sidelobe.

ally; metatibia with sparse semi-erect setae dorsally and dense appressed setae ventrally; metatibial spur absent. Forewing 1.9 times as long as broad; costal cell 0.31 times as long as wing, without setae; submarginal vein and basal area of wing bare; wing veins clear and difficult to discern beyond submarginal vein, stigma elongate oval and about 4 times as long as broad; disc of wing with microsetae ventrally. Hind wing broad and apically rounded, 3.4 times as long as broad.

Petiole 2.7 times as long as broad in lateral view, 1.6 times as long as metacoxa (Fig. 3); very slightly curved in profile; bare with fine granulate sculpture. Gaster globose, first tergite (Gt_1) 1.7 times as long as broad, smooth with few short setae dorsally; first gastral sternite smooth.

Female (Paratype; differences from male). Length 3.72 mm. Dark brown to black with faint metallic luster; antenna, mandible, petiole and basal two thirds of femora brown; rest of legs light brown.

Head 1.7 times as broad as high (Fig. 6). POL 2.5 times LOL; POL 2.8 times OOL. Frons and face finely costate (Fig. 7); clypeus smooth, supraclypeal area lightly costate. Eyes separated by 2.0 times their height. Malar space 0.77 times height of eye. Basal 7 flagellomeres with long branches ranging from 6.2 to 1.4 times as long as basal length (Fig. 6), flagellomere branches arranged linearly along flagellar axis, branches cylindrical; flagellomeres 8 and 9 lobate and with terminal segment forming a loose 3-segmented clava.

Mesosoma with areolate sculpture, interstices narrow and smooth (Figs 8, 9, and inset). Mesoscutum 2.1 times as broad as long; scutellar-axillar complex 1.1 times as long as maximum width of scutellum. Propodeal disc flat with broad alveolate sculpture medially, disc laterally areolate (Fig. 9). Metafemur 3.4 times as long as broad. Forewing 2.1 times as long as broad; costal cell 0.31 times as long as wing. Hind wing 3.4 times as long as broad.

Petiole 1.8 times as long as broad, 1.0 times as long as metacoxa; curved in profile, sculpture granulate with weak irregular carinae laterally. Gaster globose (Fig. 9), first tergite (Gt_1) 1.4 times as long as broad, smooth with few short setae dorsally; first gastral sternite longitudinally strigate with broad median keel. Hypopygium with two bristles. Ovipositor acicular.

Etymology. Named for the white antenna and light-colored petiole of the male.

Holotype: Argentina: Santiago del Estero Province, E of Lavalle, RN64, 512m, 28°09'29"S 64°55'37"W, 10.III.2007, J. and J. Heraty, chaco vegetation H07-004, UCRC_ENT 161499; deposited in UCR. Paratype: Argentina: Catamarca, Punta de Balasto, 2100m, 25–26.I.1995, P. Fidalgo, UCRC_ENT 91260 (1 female, IFML).

Phylogenetic Results

Only two genera of the Stilbula clade (*sensu* Heraty 2002) were not included in these analyses. *Neostilbula* (Madagascar) is now considered as a member of the Eucharis clade in the Eucharitini (Heraty unpublished). Specimens of *Striostilbula* (Australia) were not

available for molecular analysis, although its placement in the Stilbula clade is based on weak morphological support and its inclusion is suspect (Heraty 2002). Parsimony analysis of the 7 genera in the Stilbula clade resulted in a single tree (247 steps, r.i. 0.88; Fig. 23). Successive approximations weighting generated the same tree, suggesting the data provide stable results (Carpenter 1988). Bootstrap support was high for most nodes across the tree. The RAxML results provided nearly the same tree topology, but with *Stilbuloida* sister group to the rest of the Stilbula clade; however the RAxML bootstrap results supported a monophyletic *Stilbuloida* + *Stilbula* (100%; Fig. 23). The RAxML results also placed *P. gibbosa* as sister group to the *P. americana* clade, but with almost no bootstrap support (52% as compared to 51% for the placement on the parsimony tree (Fig. 23). For *Pseudochalcura*, the results presented in Fig. 23 are more concordant with the morphology-based phylogeny presented in Heraty (1986). Relationships within and among the other genera were the same in both results (Fig. 23).

Two groups occur within the Stilbula clade (Fig. 23): (**A**) *Leurocharis* (Australian), *Substilbula* (Australian), *Rhipipalloidea* (Indoaustralian) and *Pseudochalcura* (New World); weak parsimony bootstrap support (52%), but strong RAxML bootstrap support (99%); and (**B**) *Stilbuloida* (Australian), *Stilbula* (Old World), *Obeza* and *Lophyrocera* (both New World); strong parsimony bootstrap support (100%) but not monophyletic in RAxML. Genera in group B all share a bare callar region on the mesosoma, and usually have strong transverse carinae on the lower face. Sister group relationships between *Rhipipalloidea* + *Pseudochalcura* and *Lophyrocera* + *Obeza* have been proposed based on both morphological and molecular data (Heraty 2002; Heraty et al. 2004). *Rhipipalloidea* and *Pseudochalura* have the postgenae or hypostomae forming a complete bridge posterior to the mandibles in association with a reduction of the maxillary complex (Heraty 2002), and monophyly of *Obeza* and *Lophyrocera* share strong projections on the propodeum and callus (Heraty 2002). Based on morphology, *Leurocharis* was treated as either a sister group to all of these genera when monophyletic, or excluded when paraphyletic (Heraty 2002). Subsequent analyses of an even larger molecular data set consistently put all of these Stilbula clade genera together in a monophyletic group (Heraty unpublished).

The new species, *P. alba*, is placed together with *P. prolata* Heraty (Fig. 23, grey box). *Pseudochalcura prolata* was placed by Heraty (1986) with two other species, *P. condylus* Heraty and *P. sculpturata* Heraty, in the prolata species group based on six synapomorphies, including males having 7 basal rami on the antennal flagellomeres, the first gastral sternite striate, and lack of a metatibial spur, which are all features shared with *P. alba*. *Pseudochalcura alba* is the only male with rami on all flagellar segments, including an elongation of the terminal segment (Fig. 4). Females of *P. alba*, *P. sculpturata,* and *P. prolata* have at least the basal flagellomeres ramose (Figs 6, 14, 23g). Females of all other species of *Pseudochalcura* have either cylindrical or at most dorsally serrate antennae (Figs 10, 12, 16, 23e). Based on the lack of costal cell setae, *P. pauca* was treated as a potential sister group of the prolata group, but it has serrate, not ramose, antennae (Fig. 23f); the male is unknown (Heraty 1986). *Pseudochalcura gibbosa*, along with *P. septuosa* Heraty and *P. atra* Heraty, was proposed as the sister

group of *P. pauca* + the prolata group (Heraty 1986), which is consistent with the current results (Fig. 23). Males of the *P. americana* clade herein and *P. gibbosa* have only the basal 4–6 flagellomeres ramose (Figs 11, 13). Based on the phylogenetic hypothesis presented in Fig. 23, in males, a flagellum bearing 4–6 basal rami originating along the dorsal midline is considered plesiomorphic for the genus (Figs 11, 13), whereas a flagellum with 7–9 rami, with an alternating origin along the midline (Figs 4, 15, 17, 18) is apomorphic within *Pseudochalcura*.

Females of *Rhipipalloidea* from Australia have either simple cylindrical flagellomeres as in *R. gruberi* (Girault) (Fig. 19), dorsally serrate flagellomeres as in *R. mira* (Girault) (Fig. 20), or completely ramose flagellomeres as in several undescribed species from Sarawak (Fig. 21), Vietnam, and the Philippines. One undescribed species from Mudigere (western India) has dorsally serrate flagellomeres. Males of all species of *Rhipipalloidea* have long dorsal rami on flagellomeres 1–7 (Figs. 22, 23d). Female rami always originate along the midline, whereas male rami have alternating sites of origin. Thus the range of antennal morphology in these Australasian species parallels the diversity found in their New World sister group.

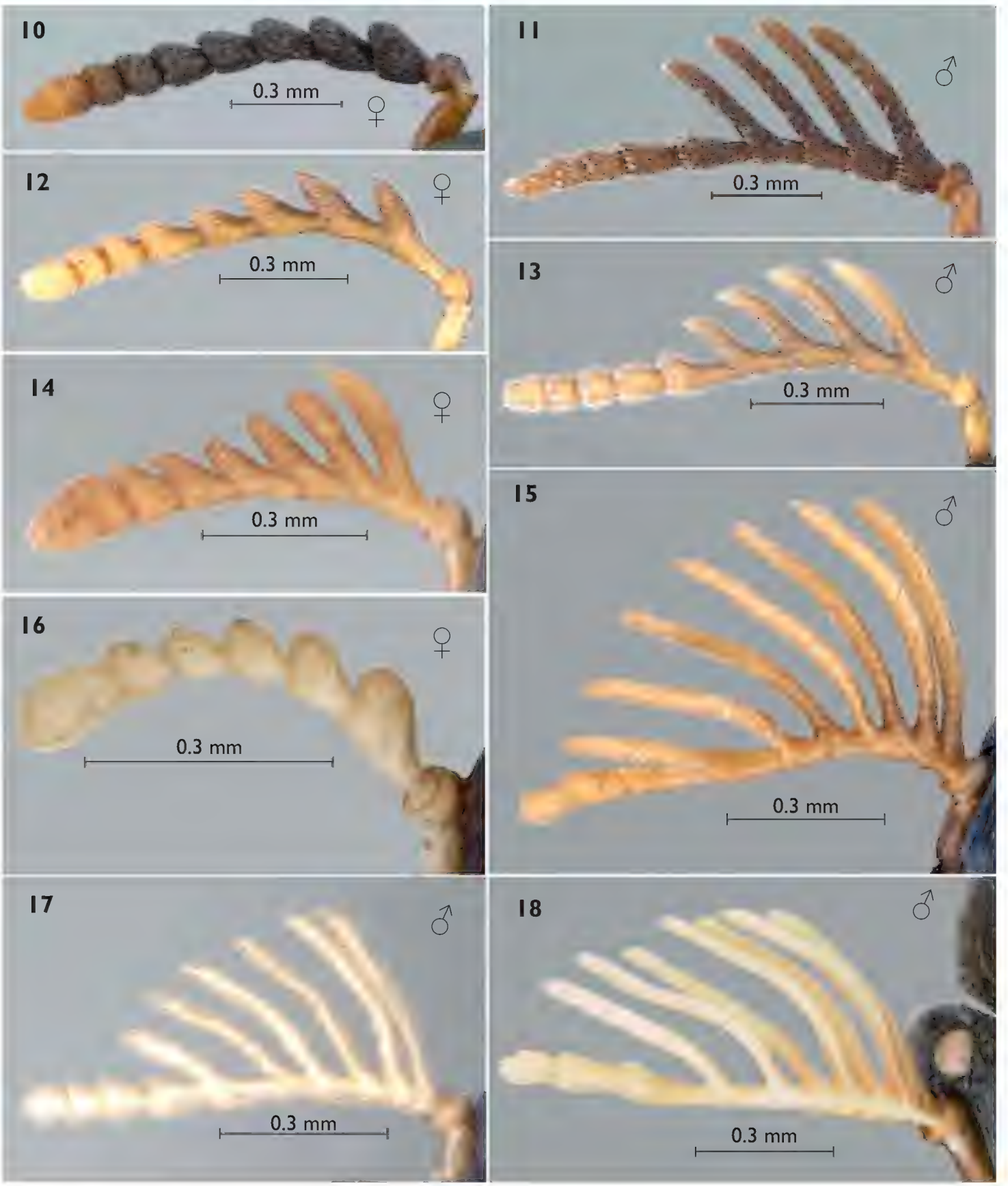
Discussion

The two groups proposed on the basis of morphological and molecular evidence not only revise our understanding of the evolutionary history of the group, but also suggest evolutionary character transitions that are of particular interest in the Eucharitidae. Four unusual evolutionary changes across the group are of particular interest within the family: coloration of the head and mesosoma, fusion of the postgenal area behind the mouthparts, a shift in oviposition habits from leaf buds to fruit, and morphological changes in the male and female antennae.

A yellow-patterned coloration of the mesosoma with contrasting black to dark green or blue patches is rare within Eucharitidae and occurs only within the Stilbula clade and some species of the eucharitine genus *Eucharis* (Heraty 2002). Among related Chalcidoidea, a similar color pattern occurs only within the distantly related Philomidinae (tentatively placed within Perilampidae). Within the Stilbula group A, only *Pseudochalcura sculpturata* Heraty (prolata group, gray box, Fig. 23; *P. prolata* not included on tree) has a yellow and black patterning of the mesosoma, with other species either brown, black, or black with faint metallic tints. Each of the genera in group B (*Stilbuloida*, *Stilbula*, *Obeza* and *Lophyrocera*) have some or all species with a black and yellow pattern on the mesosoma (Heraty 2002). Among these species, the base color of the mesosoma is almost always yellow, with a general orange color found only in *Obeza floridana* (Ashmead) (Heraty 2002). Coloration can be highly variable, with individuals of a species at a single locality ranging from almost completely black or metallic blue to mostly yellow (e.g. *Stilbula septentrionalis* (Brues), Heraty 1985; *Lophyrocera variabilis*, Torréns et al. 2008). Based on outgroup comparison across Eucharitidae, a uniformly brown, black or metallic mesosoma is plesiomorphic. Therefore a yellow

base color has developed independently as least three time in *Eucharis* and the two Stilbula clade groups, and possibly multiple times within the Stilbula clade B group.

In *Pseudochalcura* and *Rhipipalloidea*, the postgenae are completely fused as a transverse bridge posterior to their highly reduced maxillary complex. In *Substilbula*, the hypostomae are fused dorsally just below the foramen, but with the postgenae widely separated and the maxillary complex slightly reduced (fig. 338, Heraty 2002). In *Obeza*, *Lophyrocera*, *Leurocharis* and *Substilbula*, the postgenae are strongly produced



Figures 10–18. *Pseudochalcura* antennae in lateral view, sex as indicated **10–11** *P. gibbosa* **12–13** *P. nigrocyanea* **14–15** *P. sculpturata* **16–17** *P. condylus* **18** *P. prolata*.

medially and narrowly separated, but never fused and the maxillary complex is not reduced (Heraty 2002). Only in *Stilbuloida*, *Stilbula* and *Striostilbula* are the hypostomal and genal lobes broadly separated and maxillary complex fully developed as in other Eucharitidae. Mouthparts are reduced in a few other eucharitids (e.g. *Indosema indica* Husain & Agarwal and *Pseudometagea nefrens* Heraty), but without a correlated extension of the postgenae. Outside of the Stilbula clade, only *Orasema simulatrix* Gahan (Eucharitidae: Oraseminae) has the postgenae strongly produced, but without the corresponding reduction of the maxillary complex. None of the hypotheses for fusion of the postgenae in the Stilbula clade, either herein or based entirely on morphology (Heraty 2002) support a linear transformation series for the fusion of the genae from broadly separated, to closely associated, to completely fused. Instead, multiple independent origins of each of the two derived character states is supported.

Obeza and *Lophyrocera* are the only eucharitids known to oviposit small batches of eggs (60–100) under the epidermal layer of small fruits; other members of the Stilbula clade oviposit into existing cavities within leaf or flower buds (Fig. 23; Heraty

Figures 19–22. *Rhipipalloidea* antennae in lateral view, sex as indicated **19** *R. gruberi* **20** *R. mira* **21–22** *R.* sp. n. (Sabah, Malaysia), male flagellum broken beyond F4.

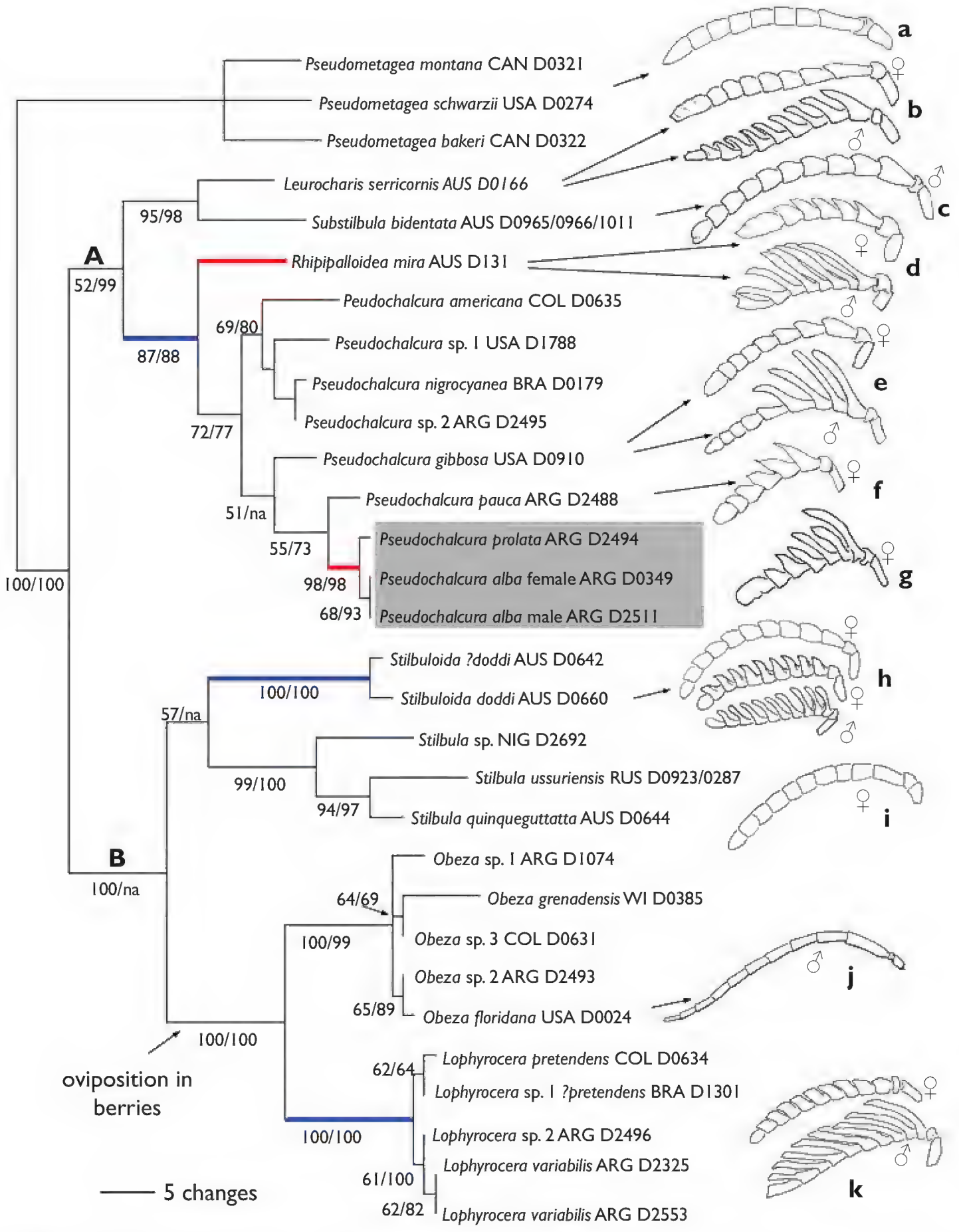


Figure 23. Most parsimonious tree of the Stilbula clade based on analysis of 18S and 28S (247 steps, r.i. 0.88); same tree recovered from RAxML likelihood analysis. Values below branches are bootstrap scores based on parsimony (1000 reps) and RAxML rbs search (200 reps). The prolata species group is shaded. Antennae (species either included or representative of taxa): a, *Pseudometagea schwarzii*; b, *Leurocharis serricornis*; c, *Substilbula bidentata*; d, *Rhipipalloidea mira*; e, *Pseudochalcura gibbosa*; f, *Pseudochalcura pauca*; g, *P. sculpturata*; h, *Stilbuloida doddi*; i, *Stilbula polyrachicidia*; j, *Obeza floridana*; k, *Lophyrocera apicalis* (figures redrawn from Heraty 1986, 2002). Red line is development of dorsal rami on female antenna; blue line is development of dorsal rami on male antenna. Clades A and B marked. Species names are followed by country codes and DNA voucher number (see Table 1).

and Barber 1990; Heraty 2002; Torréns et al. 2008). The outgroup for our analysis, *Pseudometagea,* oviposit into cavities in the seed heads of Poaceae (Heraty and Darling 1984), and most Eucharitini oviposit into flower buds or scatter their eggs on the leaf surface (Heraty 2002). Fruit oviposition and the direct association with a frugivorous ant host is unique (Heraty and Barber 1990). Across the Stilbula clade behaviors range from oviposition by *Pseudochalcura* of large masses of eggs (>1000) into leaf buds (Heraty and Barber 1990; Heraty 2002), oviposition by *Stilbuloida* into the base of nectary-filled trumpet-shaped flowers of *Loranthus* (Heraty 2002, unpublished), to a variety of behaviors in *Stilbula* such as oviposition of large masses (>10,000 eggs) into leaf buds or small masses on the side of wind-dispersed achenes (Clausen 1940). In all cases, there is either a direct association with fruit (apomorphic), an association of the eggs with ant-attractive flower nectaries, or with large egg-masses that resemble fruit and potentially attract the host ants (Heraty and Barber 1990). These are considered as apomorphic behaviors for the entire Stilbula clade, but plesiomorphic to fruit oviposition, and all involve an association with recruitment behavior and the frugivorous habits of camponotine ants (Heraty and Barber 1990).

Antennal morphology across the Stilbula clade is highly diverse. The plesiomorphic condition, as based on comparison with the other subfamilies of Eucharitidae (Gollumiellinae and Oraseminae) as well as within Eucharitinae (Psilocharitini), is a simple cylindrical flagellum (Heraty 2002). *Pseudometagea* all have a simple 8–10 segmented flagellum and have been proposed as the sister group of the remaining Eucharitini (Heraty et al. 2004; Fig. 23). Within the Stilbula clade, *Obeza, Stilbula, Stilbuloida* and *Substilbula* have simple or at most lobate flagellomeres in both sexes (Fig. 23c,i,j). *Stilbuloida doddi* is the only species with intraspecific variation in the antennae of females, ranging from simple to lobate (Fig. 23h). Basal flagellomeres of females that are either slightly or strongly lobate (Figs 10, 12, 16) are considered plesiomorphic, whereas a basally or completely ramose flagellum (Figs 6, 14, 23g) is apomorphic. The current hypothesis (Fig. 23) would suggest that long rami on the flagellomeres of females (red line) is derived independently in the Asian species of *Rhipipalloidea* and in the prolata group of *Pseudochalcura*. Ramose male antennae have developed independently at least three times in the Stilbula clade (blue lines, Fig. 23). A dorsal ramus on at least the basal flagellomeres is found in males of *Rhipipalloidea*, *Pseudochalcura*, *Stilbuloida* and *Lophyrocera*. In *Leurocharis,* the female has a simple flagellum and the male has a dorsally strongly lobate flagellum (Fig. 23b). *Striostilbula* females have simple flagellomeres, whereas males have relatively short basal flagellomeres similar to *Stilbuloida* (Heraty 2002). Females always have the projections oriented medially along the midline, as do males of *Leurocharis*, *Stilbuloidea*, *Striostilbula* and some *Pseudochalcura*. The dorsal origin of rami in males of *Lophyrocera*, *Rhipipalloidea* and some *Pseudochalcura* alternate along the dorsolateral and dorsomedial surfaces of each flagellomere. Dorsal rami with linear or alternating dorsal origins have arisen independently in males of the Eucharis (uncommonly), Chalcura (commonly), and Kapala (all species) clades, but always with the rami along all segments, and never in females.

Only *Pseudochalcura* and *Rhipipalloidea* have developed ramose antennae in females. While ramose antennae in males are common, they have developed independ-

ently in different groups of Eucharitini, and often in different forms. Across Eucharitini, there are similar differences in the origin of the rami, either medial or offset from the midline in males. The latter may be interpreted as a way of increasing the surface area for detecting pheromones. However it is not so easy to postulate why females would develop elongate rami. The question which remains is whether this is a pleiotropic effect linked to male antennal development, or a unique origin related to finding the accurate host plant for oviposition? The biology of both *Rhipipalloidea* and the prolata group of *Pseudochalcura* is unknown, and only further natural history insights from the field will likely help to resolve these issues.

There is strong support from both morphological and molecular data for a parallel split in both group A and B between the New World and Old World genera. In group A, there is a grade between the strictly Australian genera (*Leurocharis* and *Substilbula*), the Australasian *Rhipipalloidea*, and the New World *Pseudochalcura*. *Rhipipalloidea* extends north to the Philippines, whereas *Pseudochalcura* occurs as far north as Alaska and the Yukon (Heraty 1986, 2002). The chances for a recent exchange across a Beringian land connection appear likely. In group B, there is a split between the Australian *Stilbuloida* and the widespread Old World genus *Stilbula*, with the latter having an extensive northern distribution in Japan and western Russia (Heraty 2002). *Lophyrocera* and *Obeza* are restricted to the New World, with *Obeza* found only in subtropical areas of North America, but with *Lophyrocera* occurring as far north as the state of Washington in western North America. Again a recent faunal exchange seems likely. Based on molecular data, Carmichael (2003) predicted an age of divergence for the Stilbula clade ranging between 20–25 million years ago. This would fit well with a late Miocene or later dispersal event of these different groups within the Stilbula clade across the northern hemisphere (Davis et al. 2002; Renner et al. 2004).

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